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(54) Sugar-modified liposome and products comprising the liposome

(57) The present invention provides a sugar-modified liposome having a sugar chain bonded to its membrane surface, preferably through a linker protein, and having excellent absorption qualities, particularly in the intestine. The molecular structure and quantity of the sugar chain is selectively varied to allow the liposome to be delivered in a targeted manner to selected cells and tissues. The liposome is applicable to medicinal drugs, cosmetics and other various products in the medical/pharmaceutical fields, and it is especially useful in a therapeutic drug delivery system that recognizes target cells and tissues, such as cancer cells, and in the delivery of drugs or genes locally to a selected region, or in a diagnostic cell/tissue sensing probe.

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tematic research into liposomes having a wide variety of sugar chains, on the glycolipids or glycoproteins bonded to the liposomes, including preparative methods and *in vivo* analyses thereof, is pending and represents an important challenge to be progressed in future.

[0009] Further, in research on new types of DDS materials, it is an important challenge to develop a DDS material capable of being orally administered in the easiest and cheapest way. For example, when a peptide medicine is orally administered, it is subject to enzymolysis and may be only partially absorbed in the intestine due to its water solubility, high molecular weight, and low permeability in the mucosa of small intestine. As an alternative, a ligand-bonded liposome is getting attention as a potential DDS material for delivering high molecular-weight medicines or genes into the blood stream through the intestine (Lehr, C.-M. *J. Controlled Release* 65:19-29 (2000)). However, results from research into an intestinal absorption-controlled liposome, using a sugar chain as the ligand, have not been reported.

SUMMARY OF THE INVENTION

[0010] It is therefore an object of the present invention to provide a sugar-modified liposome that is specifically recognized and bound by selected lectins (sugar-recognizing proteins) residing on the surface of target cells and tissues, and having excellent qualities of absorption, particularly in the intestine. It is a further object of the present invention to provide a liposome product comprising a drug or gene encapsulated by a sugar-modified liposome that is recognized by cells or tissues *in vivo*, and that can specifically deliver drugs or genes to target cells or tissues.

[0011] In order to meet the challenges mentioned above, various experimental tests and studies have been conducted on the properties of liposome surfaces, and on the sugar chains and linker proteins used to bond the sugar chains to the surface of liposomes. Through this research, it has been shown that the targeting performance of sugar-modified liposomes to particular target tissues can be controlled by the sugar chain structure. It has also been shown that the amount of liposome transferred to each target tissue can be increased by hydrating the liposome surface and/or the linker protein, resulting in more effective delivery of drugs or genes to each of the target cells or tissues.

[0012] According to a first aspect of the present invention, there is provided a liposome having a sugar chain bonded to the liposome membrane surface.

[0013] According to a second aspect of the present invention, there is provided a liposome having a sugar chain bonded to the liposome membrane surface, and further comprising tris (hydroxymethyl) aminomethane bonded to the liposome membrane surface.

[0014] According to a third aspect of the present invention, there is provided a liposome having a sugar chain bonded to the liposome membrane surface through a linker protein.

[0015] According to a fourth aspect of the present invention, there is provided a liposome having a sugar chain bonded to the liposome membrane surface through a linker protein, wherein both the liposome membrane surface and the linker protein are hydrophilized.

[0016] According to a fifth aspect of the present invention, there is provided a liposome product comprising the sugar-modified liposome according to any one of the first to fourth aspects of the present invention, and a drug or gene encapsulated in the sugar-modified liposome.

[0017] In each aspect of the present invention, the sugar chain is preferably selected from the group consisting of lactose disaccharide, 2'-fucosyllactose trisaccharide, difucosyllactose tetrasaccharide, 3-fucosyllactose trisaccharide, Lewis X trisaccharide, sialyl Lewis X tetrasaccharide, 3'-sialyllactosamine trisaccharide, and 6'-sialyllactosamine trisaccharide.

[0018] In each aspect of the present invention, preferably an adjusted amount of the sugar chain is bonded to the membrane surface of the liposome.

[0019] In each relevant aspect of the present invention, preferably the surface of the liposome and/or the linker protein is hydrophilized. Preferably, the hydrophilization is performed by using tris (hydroxymethyl) aminomethane.

[0020] In each relevant aspect of the present invention, the linker protein is preferably human serum albumin or bovine serum albumin.

BRIEF DESCRIPTION OF THE DRAWINGS

[0021]

Fig. 1 is a schematic diagram of a liposome modified by lactose disaccharide.
Fig. 2 is a schematic diagram of a liposome modified by 2'-fucosyllactose trisaccharide.
Fig. 3 is a schematic diagram of a liposome modified by difucosyllactose tetrasaccharide.
Fig. 4 is a schematic diagram of a liposome modified by 3-fucosyllactose trisaccharide.
Fig. 5 is a schematic diagram of a liposome modified by Lewis X trisaccharide.
Fig. 6 is a schematic diagram of a liposome modified by sialyl Lewis X tetrasaccharide.

[0028] The lipid constituting the liposomes of the present invention includes phosphatidylcholines, phosphatidylethanolamines, phosphatidic acids, gangliosides, glycolipids, phosphatidylglycerols, and cholesterol. The phosphatidylcholines preferably include dimyristoylphosphatidylcholine, dipalmitoylphosphatidylcholine, and distearoylphosphatidylcholine. The phosphatidylethanolamines preferably include dimyristoylphosphatidylethanolamine, dipalmitoylphosphatidylethanolamine, and distearoylphosphatidylethanolamine. The phosphatidic acids preferably include dimyristoylphosphatidic acid, dipalmitoylphosphatidic acid, distearoylphosphatidic acid, and dicetylphosphoric acid. The gangliosides preferably include ganglioside GM1, ganglioside GD1a, and ganglioside GT1b. The glycolipids preferably include galactosylceramide, glucosylceramide, lactosylceramide, phosphatide, and globoside. The phosphatidylglycerols preferably include dimyristoylphosphatidylglycerol, dipalmitoylphosphatidylglycerol, and distearoylphosphatidylglycerol.

[0029] While a regular liposome may be used in the invention, it is preferable to hydrophilize the surface of the liposome.

[0030] The liposome itself can be produced through any conventional method including a thin film method, a reverse phase evaporation method, an ethanol injection method, and a dehydration-rehydration method.

[0031] The particle size of the liposome can be controlled through an ultrasonic radiation method, an extrusion method, a French press method, a homogenization method or any other suitable conventional method.

[0032] A specific method of producing the liposome itself of the present invention will be described below. For example, a mixed micelle is first prepared by mixing a compounded lipid consisting of phosphatidylcholines, cholesterol, phosphatidylethanolamines, phosphatidic acids, and gangliosides or glycolipids or phosphatidylglycerols, with sodium cholic acid serving as a surfactant. Particularly, the phosphatidylethanolamines are essentially compounded to provide a hydrophilic reaction site, and the composition of gangliosides or glycolipids or phosphatidylglycerols are essentially compounded to provide a bonding site for the linker protein.

[0033] The obtained mixed micelle is subjected to ultrafiltration to prepare a liposome. Then, the membrane surface of the liposome is hydrophilized by applying a bivalent crosslinking reagent and tris (hydroxymethyl) aminomethane onto the lipid phosphatidylethanolamine of the membrane of the liposome.

[0034] The liposome can be hydrophilized through a conventional method such as a method of producing a liposome by using phospholipids covalently bonded with polyethylene glycol, polyvinyl alcohol, maleic anhydride copolymer or the like (Japanese Patent Laid-Open Publication No. 2001-302686). However, in the present invention, it is particularly preferable to hydrophilize the liposome membrane surface by using tris (hydroxymethyl) aminomethane.

[0035] The technique using tris (hydroxymethyl) aminomethane has some advantages superior to the conventional method of using polyethylene glycol or the like. For example, when a sugar chain is bonded onto a liposome and the molecular recognition function of the sugar chain is utilized for bringing about the targeting performance as in the present invention, the tris (hydroxymethyl) aminomethane is particularly preferable because it is a substance having a low molecular weight. More specifically, as compared to the conventional method using a substance having a high molecular weight such as polyethylene glycol, the tris (hydroxymethyl) aminomethane is less apt to become a three-dimensional obstacle to the sugar chain and to prevent the lectin (sugar-recognizing protein) on the membrane surface of target cells from recognizing the sugar-chain molecule.

[0036] In addition, the liposome according to the present invention is excellent in terms of particle-size distribution, composition, and dispersing characteristics, as well as in long-term storage stability and *in vivo* stability, even after the above hydrophilization, and thereby is suitable for forming into and using as a liposome product.

[0037] As an example of the process for forming of a liposome hydrophilized through the use of tris (hydroxymethyl) aminomethane, a bivalent reagent is added to a liposome solution. Exemplary bivalent reagents include bissulfosuccinimidylsuberate, disuccinimidylglutarate, dithiobissuccinimidylpropionate, disuccinimidylsuberate, 3,3'-dithiobissulfosuccinimidylpropionate, ethylene glycol bissuccinimidylsuccinate, or ethylene glycol bissulfosuccinimidylsuccinate. Exemplary lipids include dimyristoylphosphatidylethanolamine, dipalmitoylphosphatidylethanolamine, and distearoylphosphatidylethanolamine. Upon combination, a reaction between the bivalent reagent and the lipid occurs so as to bond the bivalent reagent to the lipid on the membrane of the liposome. Then, the tris (hydroxymethyl) aminomethane is reacted with the bivalent reagent to bond the tris (hydroxymethyl) aminomethane to the liposome surface.

[0038] In the present invention, the sugar chain may be bonded to the liposome through a linker protein. The linker protein is first bonded to the liposome by first treating the liposome with an oxidant such as $NaIO_4$, $Pb(O_2CCH_3)_4$, or $NaBiO_3$ to oxidize the gangliosides residing on the membrane surface of the liposome. The linker protein is then bonded to the gangliosides on the liposome membrane surface by a reductive amination reaction using a reagent such as $NaBH_3CN$ or $NaBH_4$.

[0039] Preferably, the linker protein is also hydrophilized by bonding a moiety having a hydroxy group to the linker protein. For example, tris (hydroxymethyl) aminomethane may be bonded to the linker protein on the liposome by using a bivalent reagent such as bissulfosuccinimidylsuberate, disuccinimidylglutarate, dithiobissuccinimidylpropionate, disuccinimidylsuberate, 3,3'-dithiobissulfosuccinimidylpropionate, ethylene glycol bissuccinimidylsuccinate, or ethylene glycol bissulfosuccinimidylsuccinate, as discussed above.

[0040] One of the ends of a bivalent crosslinking reagent is bonded to the amino groups of the linker protein. Then,

Example 1: Preparation of Liposomes

[0052] Liposomes were prepared through an improved type of cholate dialysis based on a previously reported method (Yamazaki, N., Kodama, M. and H.-J. Gabius. *Methods Enzymol.* 242:56-65 (1994)). More specifically, 46.9 mg of sodium cholate was added to 45.6 mg of lipid mixture consisting of dipalmitoylphosphatidylcholine, cholesterol, dicetylphosphate, ganglioside and dipalmitoylphosphatidylethanolamine at a mole ratio of 35 : 40 : 5 : 15 : 5, respectively, and the lipid mixture was dissolved in 3 ml of chloroform/methanol solution. The solution was then evaporated, and the resulting deposit was dried in vacuo to obtain a lipid membrane. The obtained lipid membrane was suspended in 3 ml of a TAPS buffer solution (pH 8.4), and was subjected to a supersonic treatment to obtain a clear micelle suspension. Then, this micelle suspension was subjected to ultrafiltration by using a PM 10 membrane (Amicon Co., USA) and a PBS buffer solution (pH 7.2) to prepare 10 ml of a uniform liposome (average size of 100 nm).

Example 2: Hydrophilization of Lipid Membrane Surface of Liposomes

[0053] 10 ml of the liposome solution prepared in Example 1 was subjected to ultrafiltration by using an XM 300 membrane (Amicon Co., USA) and a CBS buffer solution (pH 8.5) to adjust the pH of the solution to 8.5. Then, 10 mg of bis (sulfosuccinimidyl) suberate (BS3; Pierce Co., USA) crosslinking reagent was added to the liposome solution. The obtained solution was stirred at 25°C for 2 hours, and subsequently stirred at 7°C for one night to complete the reaction between the BS3 and the dipalmitoylphosphatidyletanolamine of the lipid on the liposome membrane. This liposome solution was then subjected to ultrafiltration by using an XM 300 membrane and a CBS buffer solution (pH 8.5). Then, 40 mg of tris (hydroxymethyl) aminomethane dissolved in 1 ml of CMS buffer solution (pH 8.5) was added to 10 ml of the liposome solution. The obtained solution was stirred at 25°C for 2 hours, and stirred at 7°C for one night to complete the reaction between the BS3 bonded to the lipid on the liposome membrane and the tris (hydroxymethyl) aminomethane. In this manner, the hydroxyl groups of the tris (hydroxymethyl) aminomethane were coordinated on the dipalmitoylphosphatidyletanolamine of the lipid on the liposome membrane to achieve the hydrophilization of the lipid membrane surface of the liposome.

Example 3: Bonding of Human Serum Albumin (HSA) to Membrane Surface of Liposomes

[0054] Human serum albumin (HSA) was bonded to the membrane surface of the liposome through a coupling reaction method based on a previously reported method (Yamazaki, N., Kodama, M. and H.-J. Gabius. *Methods Enzymol.* 242:56-65 (1994)). More specifically, the reaction was carried out through a two-stage reaction method. That is, 43 mg of sodium metaperiodate dissolved in 1 ml of TAPS buffer solution (pH 8.4) was added to 10 ml of the liposome obtained in Example 2, and the obtained solution was stirred at room temperature for 2 hours to periodate-oxidize the ganglioside on the membrane surface of the liposome. Then, the solution was subjected to ultrafiltration by using an XM 300 membrane and a PBS buffer solution (pH 8.0) to obtain 10 ml of oxidized liposome. 20 mg of human serum albumin (HSA) was then added to the liposome solution, and the obtained solution was stirred at 25°C for 2 hours. Then, 100 µl of 2M $NaBH_3CN$ was added to the PBS buffer solution (pH 8.0), and the obtained solution was stirred at 10°C for one night to bond the HSA to the liposome membrane surface through a coupling reaction between the HSA and the ganglioside on the liposome. Then, 10 ml of HSA-bonded liposome solution was obtained through an ultrafiltration using an XM 300 membrane and a CBS buffer solution (pH 8.5).

Example 4: Bonding of Lactose Disaccharide to Human Serum Albumin (HSA) bonded on Liposome Membrane Surfaces

[0055] 50 µg, 200 µg, or 1 mg of lactose disaccharide (Wako Pure Chemical Co., Japan) was added to 0.5 ml of water solution having 0.25 g of NH_4HCO_3 dissolved therein, and the obtained solution was stirred at 37°C for 3 days. Then, the solution was filtered by using a filter of 0.45 µm to complete an amination reaction at the reduction terminal of the sugar chain and obtain 50 µg of glycosylamine compound of the lactose disaccharide. Then, 1 mg of 3,3'-dithiobis (sulfosuccinimidyl propionate) (DTSSP; Pierce Co., USA) serving as a crosslinking reagent was added to 1 ml of a part of the liposome solution obtained in Example 3. The obtained solution was then stirred at 25°C for 2 hours, and subsequently stirred at 7°C for one night. Then, the solution was subjected to ultrafiltration by using an XM 300 membrane and a CBS buffer solution (pH 8.5) to obtain 1 ml of liposome in which the DTSSP was bonded to the HSA on the liposome. Then, 50 µg of the glycosylamine compound of the lactose disaccharide was added to the liposome solution. The obtained solution was stirred at 25°C for 2 hours, and subsequently stirred at 7°C for one night. Then, the solution was subjected to ultrafiltration by using an XM 300 membrane and a PBS buffer solution (pH 7.2) to bond the lactose disaccharide to the DTSSP on the human serum albumin bonded on the liposome membrane surface. In this manner, 3 types of liposomes (2 ml each), differing in the amount of sugar chain bonded thereto (referred to as

the 3-fucosyllactose trisaccharide to the DTSSP on the human serum albumin bonded on the liposome membrane surface. In this manner, 3 types of liposomes (2 ml each), differing in the amount of sugar chain bonded thereto (referred to as 3FL-1 (50 μg), 3FL-2 (200 μg), and 3FL-3 (1 mg)), in which the 3-fucosyllactose trisaccharide is bonded to the liposome through human serum albumin (Figure 4) (total lipid mass: 2 mg, total protein mass: 200 μg, average particle size: 100 nm), were obtained.

Example 8: Bonding of Lewis X Trisaccharide to Human Serum Albumin (HSA) bonded on Liposome Membrane Surfaces

[0059] Liposomes comprising Lewis X Trisaccharide-bonded HSA on the liposome membrane surface were prepared according to the method of Example 4, with the exception that 50 μg of Lewis X trisaccharide (Calbiochem Co., USA) was used in place of the lactose disaccharide. 2 ml of the liposome (LX), in which Lewis X trisaccharide is bonded to the liposome through human serum albumin (Figure 5) (total lipid mass: 2 mg, total protein mass: 200 μg, average particle size: 100 nm), was obtained.

Example 9: Bonding of Sialyl Lewis X Tetrasaccharide to Human Serum Albumin (HSA) bonded on Liposome Membrane Surfaces

[0060] Liposomes comprising sialyl Lewis X tetrasaccaride-bonded HSA on the liposome membrane surface were prepared according to the method of Example 5, with the exception that 50 μg of sialyl Lewis X tetrasaccaride (Calbiochem Co., USA) was used in place of the 2'-fucosyllactose trisaccharide. 2 ml of the liposome (SLX), in which sialyl Lewis X tetrasaccaride is bonded to the liposome through human serum albumin (Figure 6) (total lipid mass: 2 mg, total protein mass: 200 μg, average particle size: 100 nm), was obtained.

Example 10: Bonding of 3'-Sialyllactosamine Trisaccharide to Human Serum Albumin (HSA) bonded on Liposome Membrane Surfaces

[0061] Liposomes comprising 3'-sialyllactosamine trisaccharide-bonded HSA on the liposome membrane surface were prepared according to the method of Example 6, with the exception that 50 μg of 3'-sialyllactosamine trisaccharide (Seikagakukogyou Co., Japan) was used in place of the difucosyllactose tetrasaccharide. 2 ml of the liposome (3SLN), in which 3'-sialyllactosamine trisaccharide is bonded to the liposome through human serum albumin (Figure 7) (total lipid mass: 2 mg, total protein mass: 200 μg, average particle size: 100 nm), was obtained.

Example 11: Bonding of 6'-Sialyllactosamine Trisaccharide to Human Serum Albumin (HSA) bonded on Liposome Membrane Surfaces

[0062] Liposomes comprising 6'-sialyllactosamine trisaccharide-bonded HSA on the liposome membrane surface were prepared according to the method of Example 7, with the exception that 50 μg of 6'-sialyllactosamine trisaccharide (Seikagakukogyou Co., Japan) was used in place of the 3-fucosyllactose trisaccharide. 2 ml of the liposome (6SLN), in which 6'-sialyllactosamine trisaccharide is bonded to the liposome through human serum albumin (Figure 8) (total lipid mass: 2 mg, total protein mass: 200 μg, average particle size: 100 nm), was obtained.

Example 12: Bonding of Tris (Hydroxymethyl) Aminomethane to Human Serum Albumin (HSA) bonded on Liposome Membrane Surfaces

[0063] For preparing a liposome as a comparative sample, 1 mg of 3,3'-dithlobis (sulfosuccinimidyl propionate) (DTSSP; Pierce Co., USA) serving as a crosslinking reagent was added to 1 ml of a part of the liposome solution obtained in Example 3. The obtained solution was stirred at 25°C for 2 hours, and subsequently stirred at 7°C for one night. The solution was then subjected to ultrafiltration by using an XM 300 membrane and a CBS buffer solution (pH 8.5) to obtain 1 ml of liposome in which the DTSSP was bonded to the HSA on the liposome. Then, 13 mg of tris (hydroxymethyl) aminomethane (Wako Co., Japan) was added to the liposome solution. The obtained solution was stirred at 25°C for 2 hours, and subsequently stirred at 7°C for one night. Then, the solution was subjected to ultrafiltration by using an XM 300 membrane and a PBS buffer solution (pH 7.2) to bond the tris (hydroxymethyl) aminomethane to the DTSSP on the human serum albumin bonded on the liposome membrane surface. In this process, an excess amount of tris (hydroxymethyl) aminomethane, that is 13 mg, already exists. Thus, the hydrophilization of the human serum albumin (HSA) bonded on the liposome membrane surface was simultaneously completed. In this manner, 2 ml of the liposome as the comparative sample (TRIS) in which the tris (hydroxymethyl) aminomethane is bonded to human serum albumin (Figure 9) (total lipid mass: 2 mg, total protein mass: 200 μg, average particle size: 100 nm)

Table 1: (continued)

Test Result showing Lectin-Binding Activity Inhibiting Effect of Each Type of Sugar-Modified Liposome Complex					
Liposome Complex	**Inhibiting Effect (absorbance) at each density of liposome complexes (μg protein)**				
	0.01 μg	**0.04 μg**	**0.11 μg**	**0.33 μg**	**1 μg**
3SLN	0.175	0.158	0.144	0.131	0.095
6SLN	0.256	0.245	0.233	0.200	0.151

Example 15: ^{125}I-Labeling of Each Type of Sugar-Modified Liposome through the Chloramine T Method

[0066] A chloramine T (Wako Pure Chemical Co., Japan) solution and a sodium disulfite solution were prepared at 3 mg/ml and 5 mg/ml, respectively. 50 μl of the 16 different types of hydrophilized sugar-modified liposomes prepared in Example 13, and the liposome of Example 12, were put into separate Eppendorf tubes. Then, 15 μl of ^{125}I-NaI (NEN Life Science Product, Inc. USA) and 10 μl of chloramine T solution were added thereto and reacted therewith. 10 μl of chloramine T solution was added to the respective solutions every 5 minutes. After 15 minutes from the completion of the above procedure repeated twice, 100 μl of sodium disulfite serving as a reducer was added to the solutions to stop the reaction. Then, each of the resulting solutions was placed on a Sephadex G-50 (Phramacia Biotech. Sweden) column chromatography, and eluted by PBS to purify a labeled compound. Finally, a non-labeled liposome complex was added to each of the solutions to adjust a specific activity (4×10^6 Bq/mg protein). In this manner, 16 types of ^{125}I-labeled liposome solutions were obtained.

Example 16: Measurement of Transfer Rate of Each Type of Sugar-Modified Liposome Complex to Tissues of Mice with Cancer

[0067] Using an oral sonde, 13 of the different types of ^{125}I-labeled, hydrophilized sugar-modified liposomes of Example 15 (LAC-1, LAC-2, LAC-3, 2FL-1, 2FL-2, 2FL-3, DFL-1, DFL-2, DFL-3, 3FL-1, 3FL-2, 3FL-3 and TRIS) (equivalent to 3 μg of protein per mouse) were administered to male ddY mice (7 weeks of age) which had abstained from food, except for water, for one whole day, in an amount of 0.2 ml which is equivalent to 3 μg of protein per mouse. After 10 minutes, 1 ml of blood was taken from descending aorta under Nembutal anesthesia. Then, ^{125}I-radioactivity in the blood was measured with a gamma counter (Aloka ARC 300). Further, in order to check the *in vivo* stability of each type of liposome complex, serum from each mouse's blood was subjected to chromatography using a Sephadex G-50. As a result, most of the radioactivity in each sample of serum was found in void fractions having a high molecular weight, and it was proved that each type of liposome complexes has a high *in vivo* stability. The radioactivity transfer rate from intestine to blood was represented by the ratio of the radioactivity per ml of blood to the total of given radioactivity (% dose /ml blood). This measurement result is shown in Figures 10 to 13.

Example 17: Measurement of Distribution Rate of Each Type of Sugar-Modified Liposome Complex to Tissues of Mice with Cancer

[0068] Ehrlich ascites tumor (EAT) cells (about 2×10^7 cells) were implanted subcutaneously into the femoral region in male ddY mice (7 weeks of age), and the mice were used in this test after the tumor tissues grew to 0.3 to 0.6 g (after 6 to 8 days). Five of the different types of ^{125}I-labeled, hydrophilized sugar-modified liposome complexes (LX, SLX, 3SLN, 6SLN and TRIS) of Example 15 were injected into the tail veins of the mice in an amount of 0.2 ml which is equivalent to 3 μg of protein per mouse. After 60 minutes, tissues (blood, liver, spleen, lung, brain, inflammatory tissues around cancer, cancer and lymph node) were extracted, and the radioactivity of each of the extracted tissues was measured with a gamma counter (Aloka ARC 300). The distribution rate of the radioactivity in each of the tissues was represented by a ratio of the radioactivity per gram of each of the tissues to the total of given radioactivity (% dose /g tissue). This measurement result is shown in Figures 14 to 21.

[0069] The results from these experiments show that the sugar-modified liposomes of the present invention are innovative in that they are excellent in intestinal absorption and are capable of being administered via the intestine, which has not been found in conventional liposome related products. In addition, the intestinal absorption can be controlled by adjusting the identity and amount of the sugar chain bonded to the liposomes.

[0070] Furthermore, the *in vivo* mobility of sugar-modified liposomes of the present invention, and their ability to target selected tissues *in vivo*, can be facilitated or suppressed in a living body by utilizing the difference in the molecular structure of the sugar chain, and varying their amounts.

[0071] Thus, the sugar-modified liposomes of the present invention can be used to deliver drugs or genes through

FIG. 1

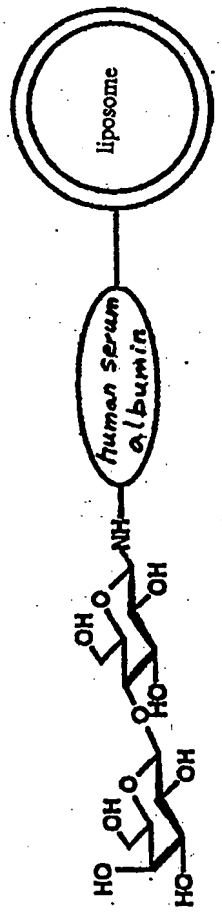


FIG. 3

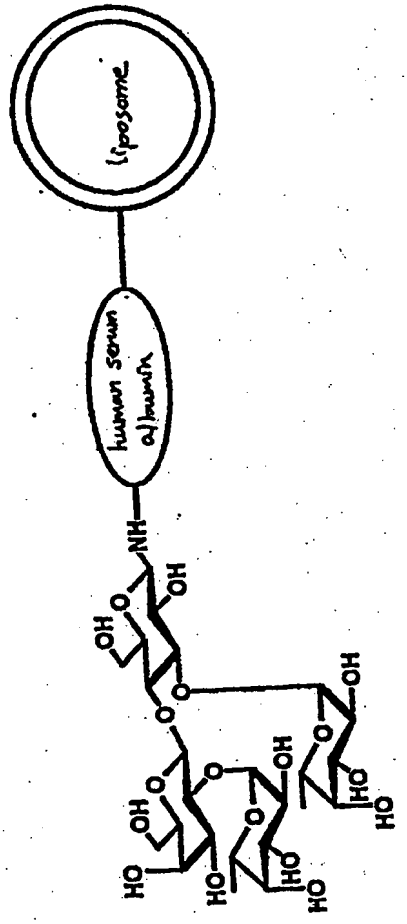


FIG. 5

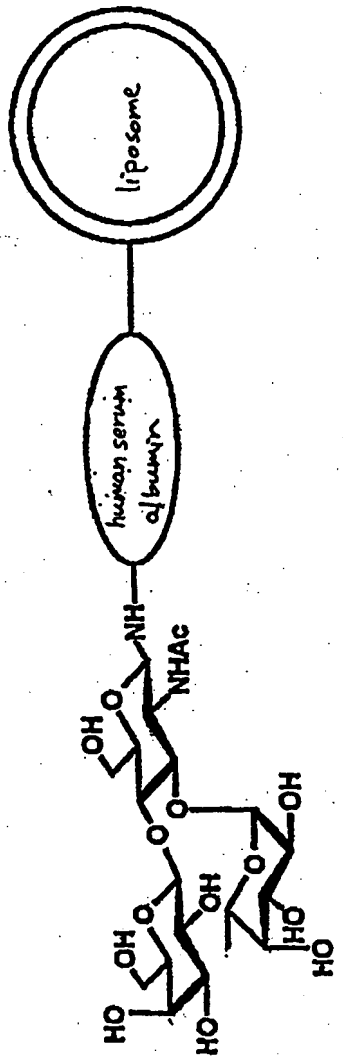
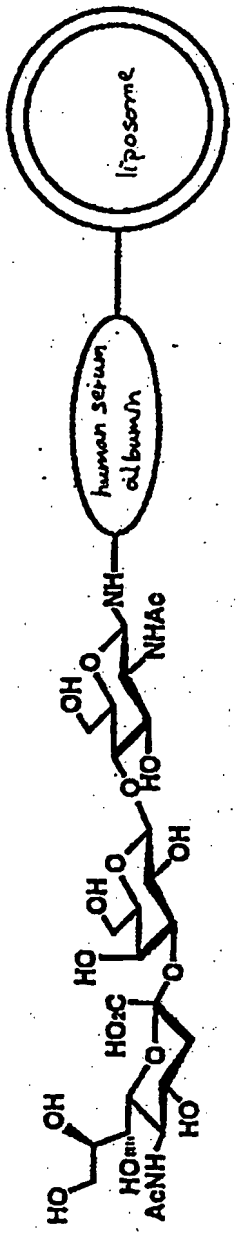


FIG. 7


liposome
human serum albumin
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NHAc
OH
O
HO
OH
HO
HO2C
HO
AcNH
HO

FIG. 9

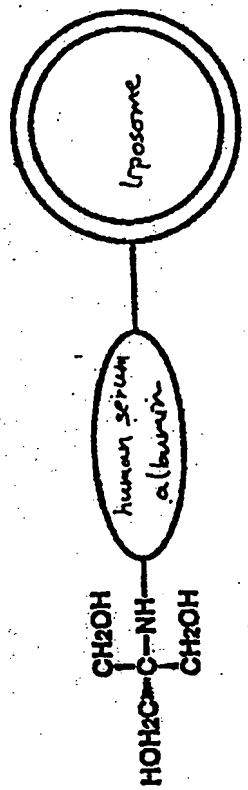


FIG. 11

transfer rates to blood of 4 types of liposome complexes after 10 minutes from their intestinal administrations

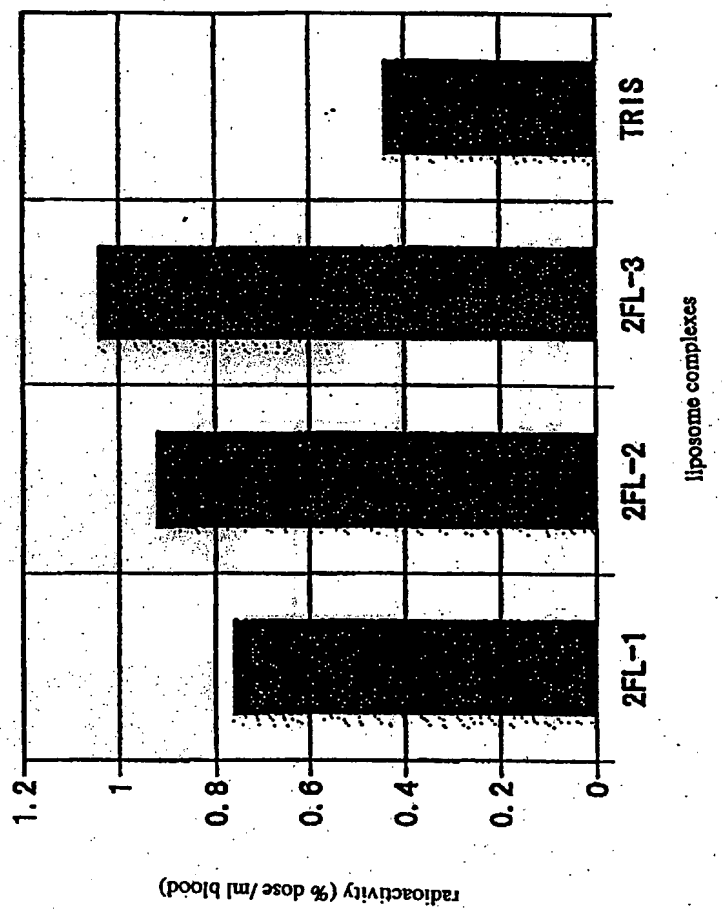


FIG. 13

transfer rates to blood of 4 types of liposome complexes after 10 minutes from their intestinal administrations

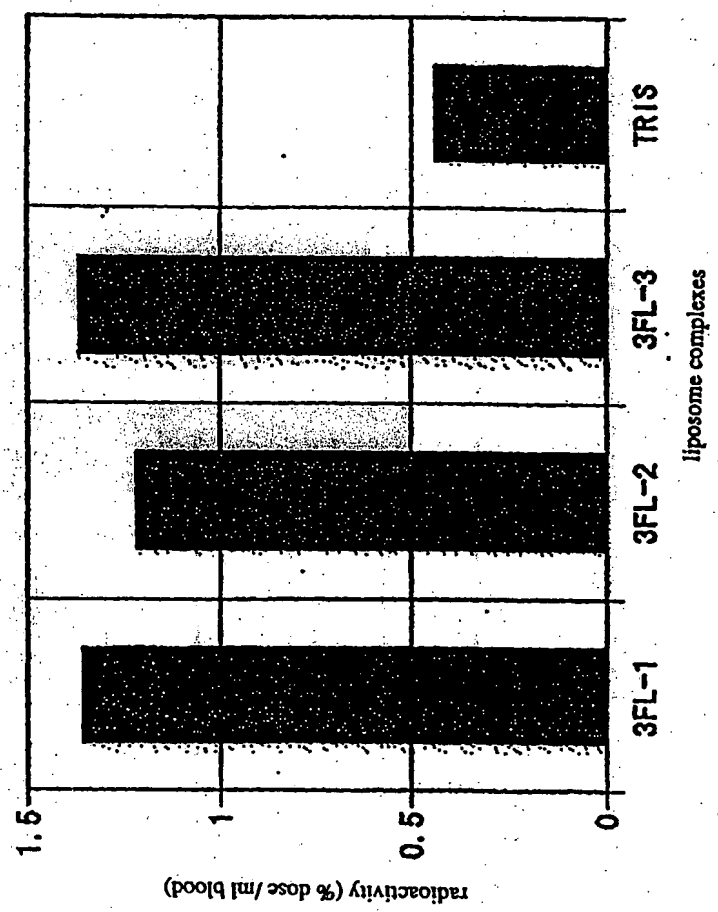


FIG. 15

distribution rates in liver of 5 types of liposome complexes after 60 minutes from their intravenous administrations

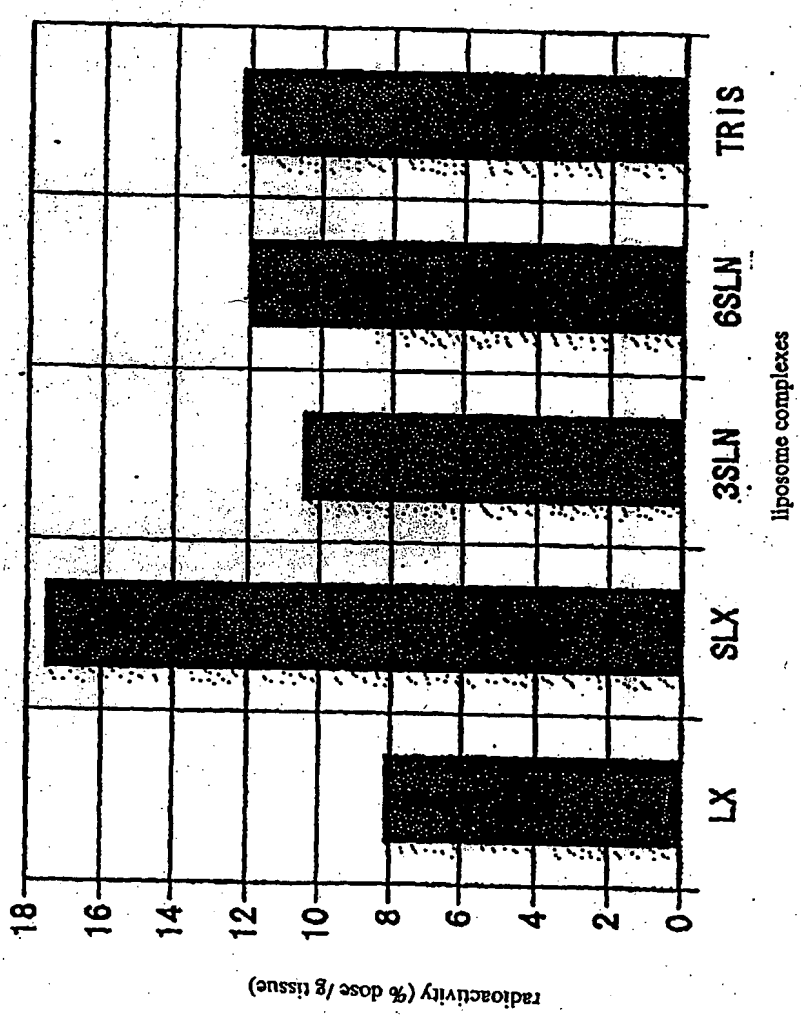


FIG. 17

distribution rates in lung of 5 types of liposome complexes after 60 minutes from their intravenous administrations

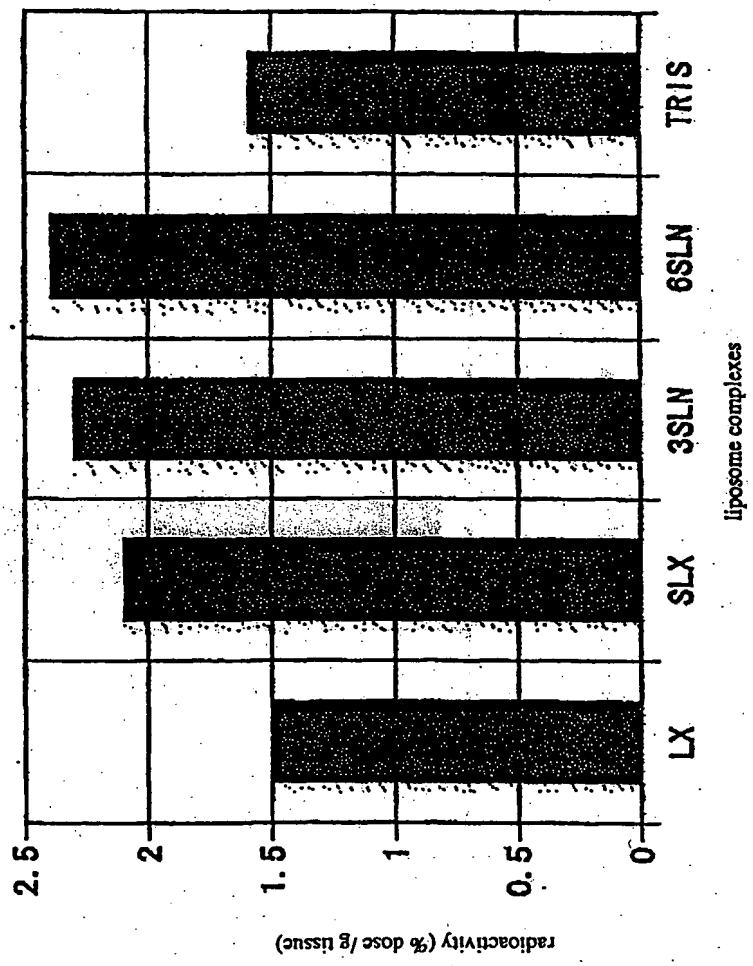


FIG. 19

distribution rates in cancer tissues of 5 types of liposome complexes after 60 minutes from their intravenous administrations

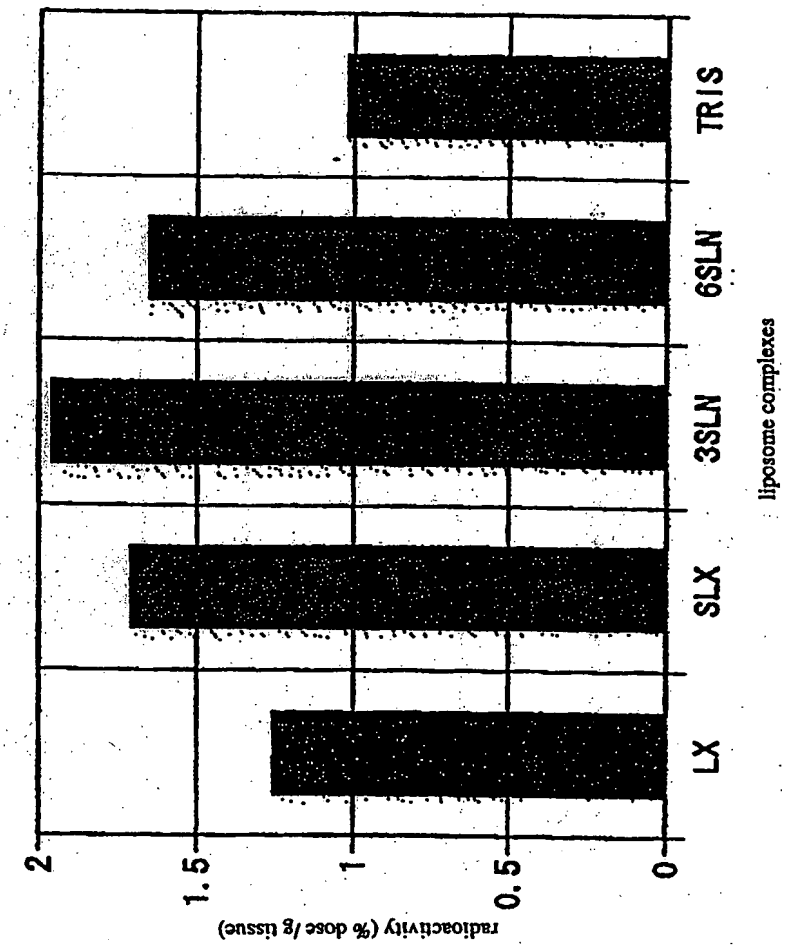
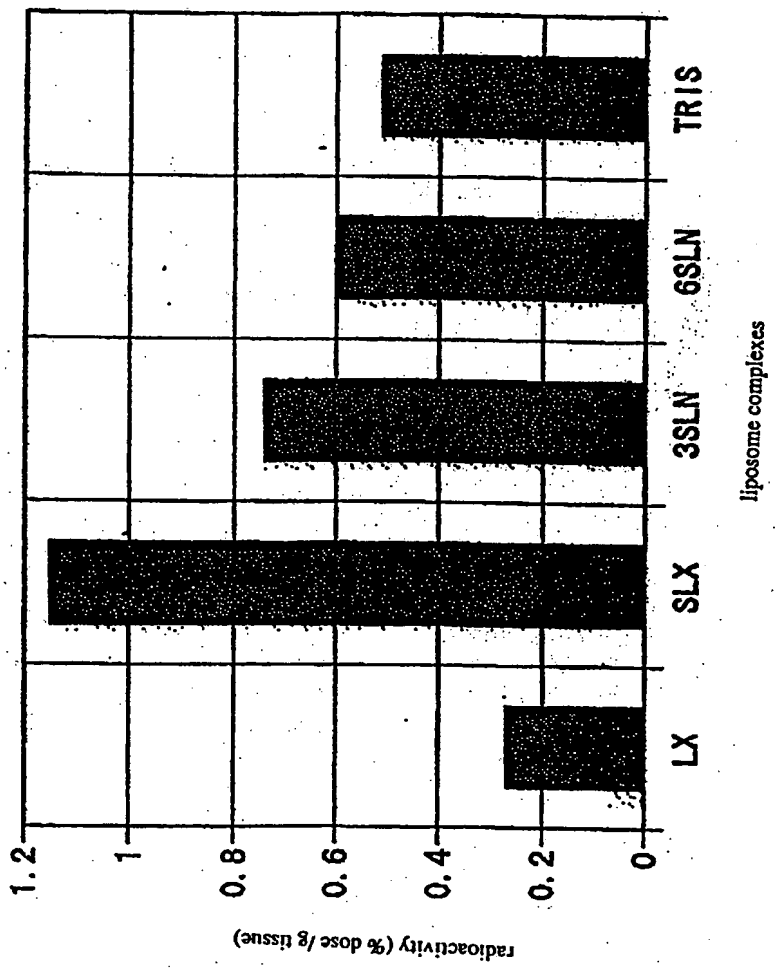


FIG. 21

distribution rates in lymph node of 5 types of liposome complexes after 60 minutes from their intravenous administrations



European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 03 25 0952

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
X	DEOL P ET AL: "Lung specific stealth liposomes: stability, biodistribution and toxicity of liposomal antitubercular drugs in mice" BBA - GENERAL SUBJECTS, ELSEVIER SCIENCE PUBLISHERS, NL, vol. 1334, no. 2-3, 15 March 1997 (1997-03-15), pages 161-172, XP004276410 ISSN: 0304-4165 * paragraph [02.4] * -----	1,5,7	

TECHNICAL FIELDS SEARCHED (Int.Cl.7)

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	24 July 2003	VON EGGELKRAUT, S

CATEGORY OF CITED DOCUMENTS

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